

MICROBIOLOGY OF AERATED LAGOONS: OXYGEN AS A
CATABOLIC REGULATOR FOR THE STABILIZATION OF
SELECTED SUBSTRATES

by

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G E N E R A L I N T R O D U C T I O N

GENERAL INTRODUCTION

The initial development and operation of artificially aerated sewage lagoons has fallen chiefly in the realm of engineering technology. Fundamentally, such aerated systems have been designed to provide for the introduction of oxygen into lagoon basins in order to meet the biological requirements of the resident microflora. In many instances, high rates of aeration have been employed with little regard for the actual biological oxygen requirements of the particular lagoon. Of practical importance, is the consideration that a reduced aeration rate could possibly be employed in some instances, without consequent losses in lagooning efficiency.

This present study involving the aerated installations of the Charleswood Sewage Lagoon was prompted by the concern that possibly oxygen was being supplied in excess of process demands. Experiments were carried out to determine the minimum dissolved oxygen levels at which rates of oxidation on some selected substrates remained optimum. Bacterial cell suspensions utilized in this study were prepared from samples taken from aerated lagoon cells. Substrates used were linear alkylate sulfonate (LAS) type detergents, benzoic acid, salicylic acid and butyric acid.

H I S T O R I C A L

HISTORY

In the last twenty years the use of artificial aeration in waste water treatment has increased markedly, and many new aeration treatment plants have been constructed and numerous existing plants have installed or improved their aeration apparatus. Since 1952 (33) innovations in aeration have been employed extensively in the activated sludge process, and more recently for the aeration of sewage lagoons (waste stabilization units). However, the fundamental uses of aeration still remain the same; firstly to furnish oxygen for the resident microflora and secondly, to mix the contents of the aerated system.

Experiments on aeration of waste water began in England as early as 1882 (30) and culminated in the development of the activated sludge process by Arden and Lockett (31) in 1914. In the earliest stages of development, the introduction of air was achieved through open or perforated tubes. Later, as greater air economy was sought, smaller bubbles were produced by means of porous diffuser equipment. However, clogging of diffusers due to sedimentation and dirty air supplies, was one of the earliest problems encountered and is still evident today (15). One solution to the clogging problem was the introduction of mechanical surface aerators;

another was the ultrafiltration of the air which was being fed through diffuser systems.

The fundamental principles of activated sludge operation have been outlined by Chasick (13). The activated sludge process is basically one wherein waste water is aerated in the presence of a large quantity of the activated sludge "floc" for a suitable time interval. The mixture is then allowed to settle in a settling tank until the solids (organisms and suspended matter) flocculate, settle out, and are partially recovered for reutilization as seed inoculum in the aeration tank. An effectively operating plant should remove approximately ninety percent or more of the suspended solids and biological oxygen demand (BOD) load in the raw wastewater. Although the variations in activated sludge processes are almost unlimited, the fundamental principles of operation are the same as those that have been presented.

Conventional oxidation or stabilization ponds (32) are expansive sewage treatment cells which are rarely more than 5 feet in depth. Such cell design allows for the penetration of light so that algal blooms may develop and introduce oxygen into the system. As well, oxygen enters the cell due to the natural exchange which occurs at the air-water interface. Stabilization is effected by the microflora indigenous to

the lagoon cell. As the BOD handling capacity/volume/day is relatively low, large areas of land and relatively long retention times are required to enable these ponds to handle sewage loads effectively.

Aerated lagoons (32, 34) are a relatively recent development resulting from attempts to augment the supply of oxygen for purposes of alleviating existing nuisance odor problems in heavily loaded oxidation ponds, increasing the rapidity and efficiency of BOD removal, and permitting a more economical use of land in the design of new units. An aerated lagoon (33) differs from an oxidation or stabilization pond by having an artificial external source of introduced oxygen. It differs from a conventional activated sludge operation in that the waste water retention time exceeds 24 hours and there is no sludge return. Furthermore, mixing is sufficient to distribute the oxygen through the basin but not sufficient to keep all the solids in suspension.

Natural waters receive their oxygen supply by exchange with the atmosphere and by photosynthesis of green aquatic flora. Air (29) is approximately 20 percent oxygen by volume and is 21 percent on a weight basis. The maximum solubility of atmospheric oxygen in water is dependent primarily upon pressure, temperature and salt content.

The solubility of molecular oxygen in water follows Henry's Law up to a pressure of 4 atmospheres of oxygen. At 25°C, its solubility in water at an oxygen partial pressure of 154 mm of mercury (air pressure of 1 atmosphere) is 8.11 mg per liter. The effect of hydrostatic pressure on oxygen solubility is essentially nil, in contrast to nitrogen which decreases in solubility with increasing water depth. As with most gases, the solubility of oxygen in water decreases with increasing temperature. At 1 atmosphere of air oxygen solubility in water at 0°C is 14.74 mg/liter, whereas at 35°C it is 7.03 mg/liter. The solubility of oxygen in water is usually decreased by the addition of other solutes particularly electrolytes. The extent to which this occurs depends upon the nature and amount of the salt present.

The role of oxygen, or more specifically dissolved oxygen (DO), on the metabolic activities of microorganisms has been a field of much investigation and controversy. It has been shown that there are critical DO tensions below which microbial metabolic activity becomes a function of the oxygen tension of the suspending media. The values for critical DO tensions reported for various microorganisms (45) cover a rather narrow range from 0 to 15 mm Hg (0 to 0.7 mg/l dissolved oxygen (DO) at 30°C). Carter and Bull (12) define the critical oxygen tension as that point at which the oxygen

uptake rate is no longer independent of oxygen tension. Below such a point, 1.75 mm Hg (0.08 mg/l DO at 30°C) in their studies with Aspergillus nidulans, they not only found a reduced respiration rate, but also alterations in other metabolic activities and capabilities such as nitrite accumulation. Harrison and Pirt (20) studied the influence of dissolved oxygen concentration on the metabolism of growing Klebsiella aerogenes NCTC 8017 employing a continuous flow culture technique. They found that the respiration rate (oxygen uptake rate per unit mass organism) was independent of DO tensions above 10-15 mm Hg (0.46-0.7 mg/l DO at 30°C). They called this "the excess oxygen state". Below about 10 mm Hg the "limited oxygen state" developed, wherein the respiratory rate became dependent upon DO tension.

The original concept of critical oxygen tension as proposed by Gerald and Falk (18) may be stated as: the tension below which the oxygen uptake rate of an organism decreases with a decrease in oxygen tension. In their work with Klebsiella aerogenes, Harrison and Pirt (20) have shown that the response to decreased oxygen tension may in some cases involve an increase in respiration rate confirming the work of White (49) and others. Harrison and Pirt therefore offered a modified definition of critical oxygen tension: the oxygen tension above which the respiration

rate of an organism is independent of changes in DO tensions; below this tension, the oxygen uptake rate of the organism may increase or decrease in oxygen tension, according to cultural conditions. The authors suggest that perhaps the increased oxygen uptake, as witnessed at low oxygen concentrations, is due to decreased ATP production yield possibly due to an alternate electron transport pathway or to partial uncoupling of oxidative phosphorylation.

In the majority of cases, however, lowered DO tensions correspond to lowered oxygen uptake rates and a number of investigators have studied the etiology of this phenomenon. Longmuir (26) suggests that with larger organisms, such as yeast cells, and Bacillus megaterium, there is a significant concentration gradient between the bulk of the liquid and the location of the respiratory system. In other words the diffusion of oxygen through the cell material to oxidative sites was rate limiting. Winzler (50) in working with bakers' yeast believed the important factor limiting respiration at low oxygen tensions to be the rate of combination of oxygen with oxygen transferring enzyme presumably cytochrome oxidase. Borkowski and Johnson (4) have studied the possibility of liquid film resistance to oxygen transport to microbial cells. In their work with yeast and bacteria they concluded that as long as the

agitation level of the medium was sufficient to keep the cells suspended, the liquid film resistance was of negligible consequence in most systems.

In a recent article, Johnson (23) has reviewed many of the controversial factors on the regulation of oxygen uptake by microorganisms. In the usual case, oxygen uptake rate at high oxygen concentrations is independent of oxygen tension. At these higher levels of dissolved oxygen, the factor which is generally thought to limit the rate of oxygen uptake has been termed the "oxygen demand of the cell". This so-called oxygen demand factor is regulated in part by the supply of intermediary metabolites such as ADP and reduced NAD. As well, the uptake rate may be limited by the concentration of oxygen reducing enzyme(s) which would restrict the cells' capacity for oxygen utilization.

Should the oxygen level be allowed to fall below the critical value for a particular microbial system then the oxygen uptake rate becomes dependent upon the oxygen tension and in most cases (exceptions have been previously documented), the oxygen uptake rate will decrease with lowering of the DO. If there is no concentration gradient, (ie. no resistance to diffusion) then the concentration of oxygen at the sites of utilization is the same as that in the bulk of the media. The lowered oxygen utilization rate can then be attributed

to a lowering of reaction velocities due to desaturation of oxygen-using enzyme(s) to such a point where the rate of catalysis is less than required to satisfy the oxygen demands of the cell. Should however it be assumed that resistance to oxygen diffusion from the bulk of the medium to the enzyme surface exists then oxygen uptake at very low oxygen concentrations will be diffusion-limited. That is, due to a concentration drop across a region of resistance, the rate of diffusion of oxygen to sites of utilization may become lessened as the oxygen concentration in the bulk of the media falls and this would ultimately be responsible for reduced rates of oxygen uptake.

As yet the exact cause of decreased oxygen uptake rates at low oxygen concentrations is generally unresolved. More recent work (4, 23) seems to stress diffusion-limited respiration with the resistance factor residing in a low intracellular diffusion barrier.

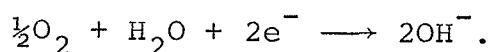
In the field of water pollution control the aerobic treatment of waste materials has been reviewed by Smith (40), Eckenfelder and O'Connor (14) and Simpson (39). Previously reported minimum DO levels recommended for operation of activated sludge plants have ranged from 0.5 ppm to 2 ppm. The minimum DO level of operation

suggested for the Air Aqua and Surface Aerator systems of the Charleswood Sewage Lagoon is 2 ppm (15). Gaudy and Turner (17) maintain that the DO tension at which bacterial respiration becomes oxygen limited and the rate of BOD removal is decreased lies in a region below 0.5 ppm oxygen tension.

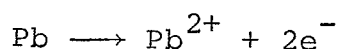
The recent development of a reliable oxygen probe and of a system whereby dissolved oxygen levels could be automatically controlled has allowed experimental work to be carried out on the significance of oxygen as a control parameter in metabolic processes. In 1964, Mackereth (1) described an improved membrane bound galvanic cell for the determination of oxygen concentrations in fluids. Until this time, many solid electrode systems for the measurement of DO had been developed (46, 27, 35). These early electrodes were not very stable for extended periods of time because the system often becomes contaminated through the deposition of oxidized material on the exposed electrode surface. Prior to 1964, membrane bound electrodes permeable only to oxygen had been described (11), but these electrodes produced output currents of a relatively low magnitude and also suffered from a lack of long-term stability.

The Mackereth electrode, named after its designer, is a membrane bound galvanic cell selectively permeable to

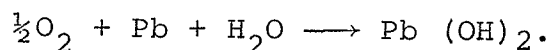
oxygen. It features an electrical output in the order of several hundreds of microamps and offers stable sensitivity over many months of operation. The working components of the galvanic cell consist of a perforated silver cathode and a solid lead anode insulated from each other by a gap containing a membrane of porous polypropylene. The anode and the cathode are bathed in an electrolytic solution which provides a path for ionic conductance. When the silver and lead electrodes are connected via the external circuit, electrons pass from the lead electrode to the silver electrode. When oxygen is present at the surface of the silver electrode, it reacts with electrons to produce hydroxyl ions as follows:



At the lead electrode the loss of electrons produces lead ions in the reaction:



The hydroxyl ions combine with the lead ions to precipitate lead hydroxide on the lead electrode. The overall cell reaction is therefore:



The current flowing in the external circuit is directly proportional to the oxygen concentration in the fluid in which the electrode assembly is immersed.

In 1966 a system for the automatic control of DO

concentration in stirred microbial cultures was described by MacLennan and Pirt (28). Equipment was developed for the automatic control of dissolved oxygen concentration by using the Mackereth electrode as a sensing probe. The equipment provided precise and reliable control over a wide range of DO tensions from 0.26 mm Hg to 30 mm Hg O_2 tension (0.01 - 1.4 mg/l DO at 30°C) and retained a high degree of stability over long periods (up to 5 weeks) of continuous use.

In 1967, experimental aerated lagoon systems were installed at the Charleswood Lagoon to test their performance against existing treatment facilities. Early operational characteristics provided for maintenance of dissolved oxygen levels in excess of 7 ppm in the Surface Aerator basin and in excess of 3 ppm in the Air Aqua basin, (figures presented represent an average of DO readings taken over a 21 month period from January 1968 to September 1969). Whether or not these seemingly high levels of dissolved oxygen were beyond process demands and not appreciably adding to the operational efficiency was unknown. This present study on oxygen as a catabolic regulator of the metabolism of a selected number of substrates was undertaken to investigate this matter.

M A T E R I A L S A N D M E T H O D S

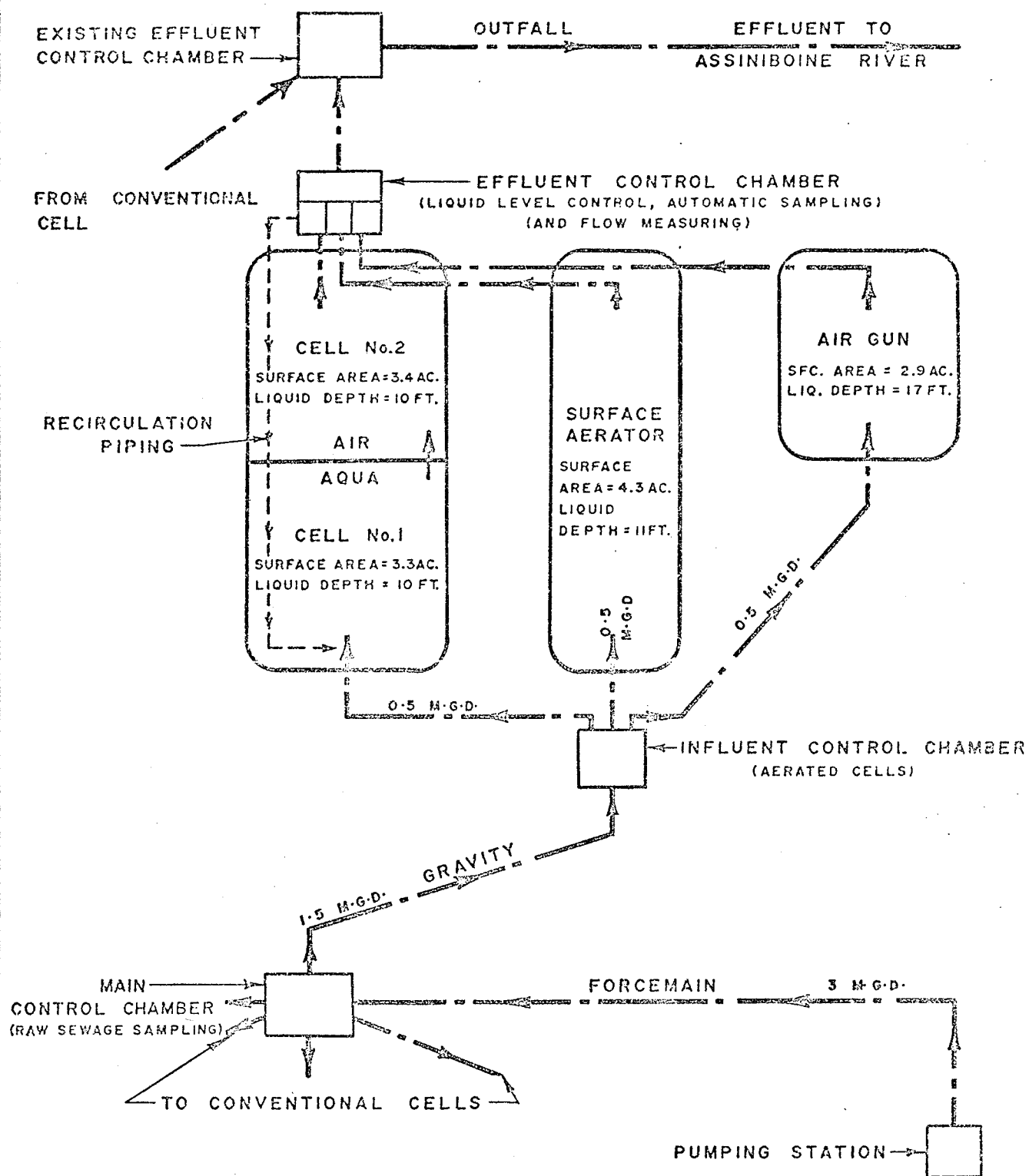
MATERIALS AND METHODS

Description of the Lagoon

In December 1967 experimental aeration systems were installed at the Charleswood Lagoon to determine their effectiveness and economy in comparison to unaerated lagoons and activated sludge treatment. Three lagoon basins were constructed, each equipped with a different type of commercially available aeration unit. A schematic diagram of the lagoon system is given in Fig. 1 and an aerial photograph is shown in Fig. 2. Two aeration systems were examined; the Air Aqua (Hinde Manufacturing Limited, Hamilton, Ont.), and the Mechanical Surface Aerators (Greedy Mixing Equipment Ltd., Toronto, Ont.).

The Air Aqua installation operates on the diffused air principle whereby air from a compressor is delivered to plastic aeration tubing laid on the bottom on the basin in a grid pattern. Air is released from the tubing by means of openings in the tubes. The tubing is spaced in the grid pattern such that maximum aeration occurs near the inlets. A 30 HP compressor supplies the air, approximately 590 cfm at 6 psi. The total length of aeration tubing is approximately 28,000 ft. with spacing between lines varying from 2.5 ft. to 20 ft. The Air Aqua basin is divided into

Fig. 1 Schematic showing the physical relationship
between the three aerobic installations
found at the Charleswood Sewage Lagoon.



NOTE

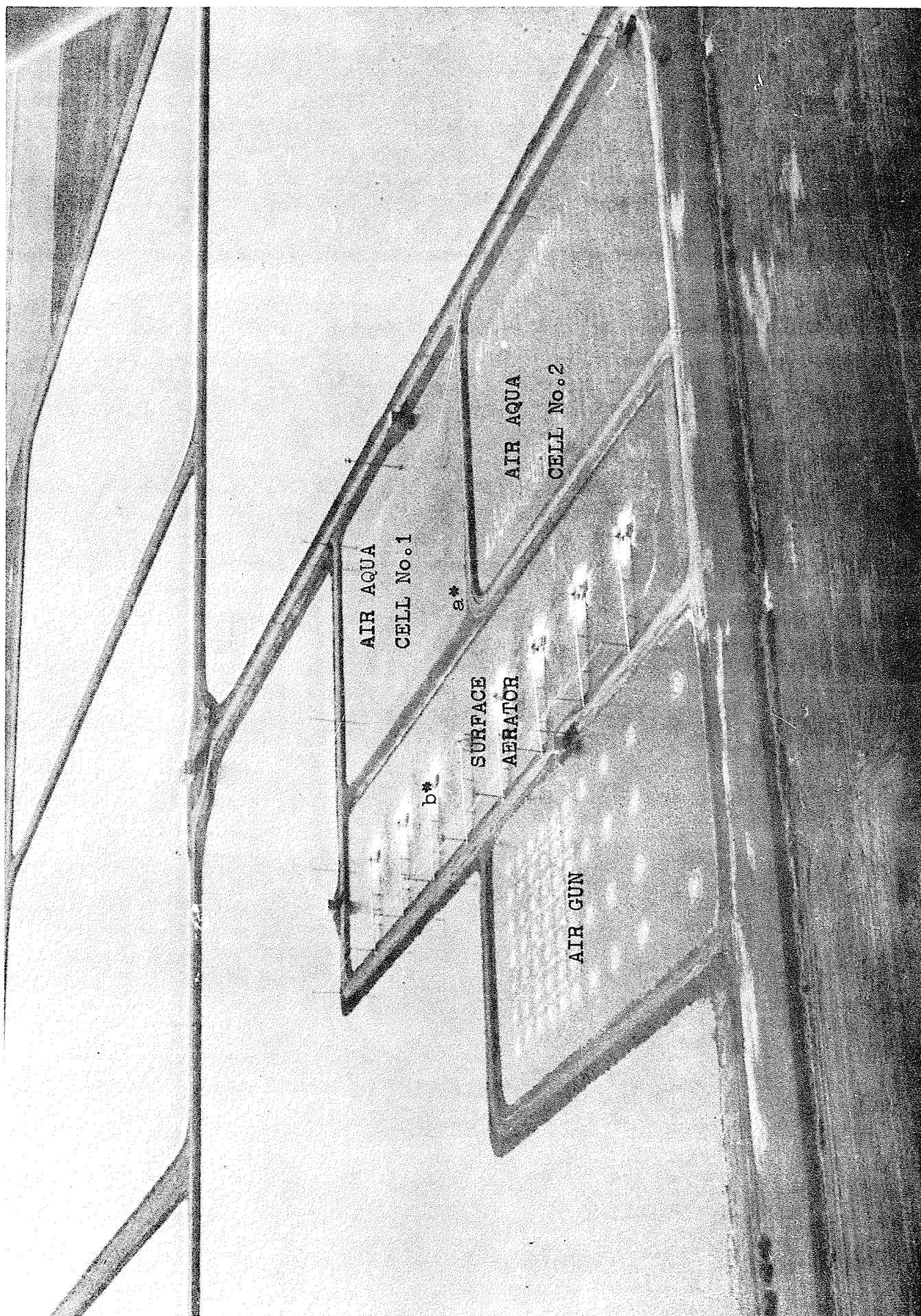
M.G.D. — IMPERIAL UNITS

SCHEMATIC OF AERATED
LAGOON PROCESSES

Fig. 2 Aerial view of aerobic installations at the Charleswood Lagoon.

a* Summer sampling site

b* Winter sampling site



2 cells for series operation.

The Surface Aerator installation consists basically of an impeller connected to a speed reducer and electric driver. When the impeller spins at the surface of the liquid it shears the liquid, creating a multitude of air bubbles, and at the same time tosses the liquid into the air where it is aerated by contact with the atmosphere. In addition each aerator provides pumping action for mixing and circulation throughout the basin. A total of 8 aerators, 20 HP each are installed in series in the lagoon basin each being mounted on a open steel deck platform set on precast piles. A summary of design data of both the Air Aqua and Surface Aerator systems is presented in Table I.

Sampling Procedure

Lagoon water was collected during the summer period from the effluent end of the Air-Aqua cell No. 1 using a Briggs and Stratton four cycle centrifugal pump. The summer sampling locale was kept constant by means of the pump hosing which was permanently fixed six inches below the water surface; the position was marked by a buoy.

During winter periods where fast freezing negated operation of the pump, samples were taken manually from

TABLE I

Summary of Design Data:
Air Aqua and Surface Aerator Systems*

Item	Value and Units
Geographic Location	50°N latitude
Average Design Flow	0.5×10^6 gallons/day
Influent 5-Day BOD 20°C	250 mg/l
Influent Suspended Solids 20°C	180 mg/l
Minimum Dissolved Oxygen	2.0 mg/l
Mean Ambient Air Temperature	
-winter	-16°F
-summer	+75°F
Treatment Efficiency Required	90%
Retention Time	
-Air-Aqua	30 days
-Surface Aerator	20 days
Operating Depth	
-Air-Aqua	10 ft
-Surface Aerator	11 ft
Volume	
-Air-Aqua	15×10^6 gal.
-Surface Aerator	10×10^6 gal.
Mixing Requirements for surface aerators	0.016 HP/1000 gals.
Process Loading	
-Air Aqua	0.52 lbs. BOD ₅ /1000 ft ³ /day
-Surface Aerator	0.78 lbs. BOD ₅ /1000 ft ³ /day

* Specifications supplied by the Metropolitan Corporation of Greater Winnipeg, Waterworks and Waste Disposal Division (15).

the dock above the Surface Aerator cell. Sampling locations for both summer and winter are indicated in Fig. 2.

Preparation of Resting Cell Suspensions

The indigenous bacterial population recovered from aerated lagoon cells was used to study the physiological activity of lagoon bacteria as a function of oxygen tension. Summer samples provided a source of mesophilic organisms whereas winter samples gave rise to psychrophiles. The method of sampling has already been described above.

The procedure adopted for preparation of resting cell suspensions is essentially the same as the method developed by Ishaque (22). Organisms in a sample of lagoon water were removed by centrifugation using a steam driven high speed Sharples Super Centrifuge (Sharples Centrifuges Limited, Camberly, Surrey) operating at twenty pounds steam pressure (approx. 40,000 rpm). When the supernatant liquid was examined microscopically, no cells could be seen. The packed sediment consisting of algae, protozoa, bacteria and clay like deposits was washed from the rotor with .05M potassium phosphate buffer, pH 7.0 and stirred on a mechanical stirrer. Algae and protozoa were then separated from the bacteria by low speed centrifugation ($365 \times g$) for ten minutes using a Sorvall centrifuge RC-2B (Ivan Sorvall, Inc., Norwalk, Conn.).

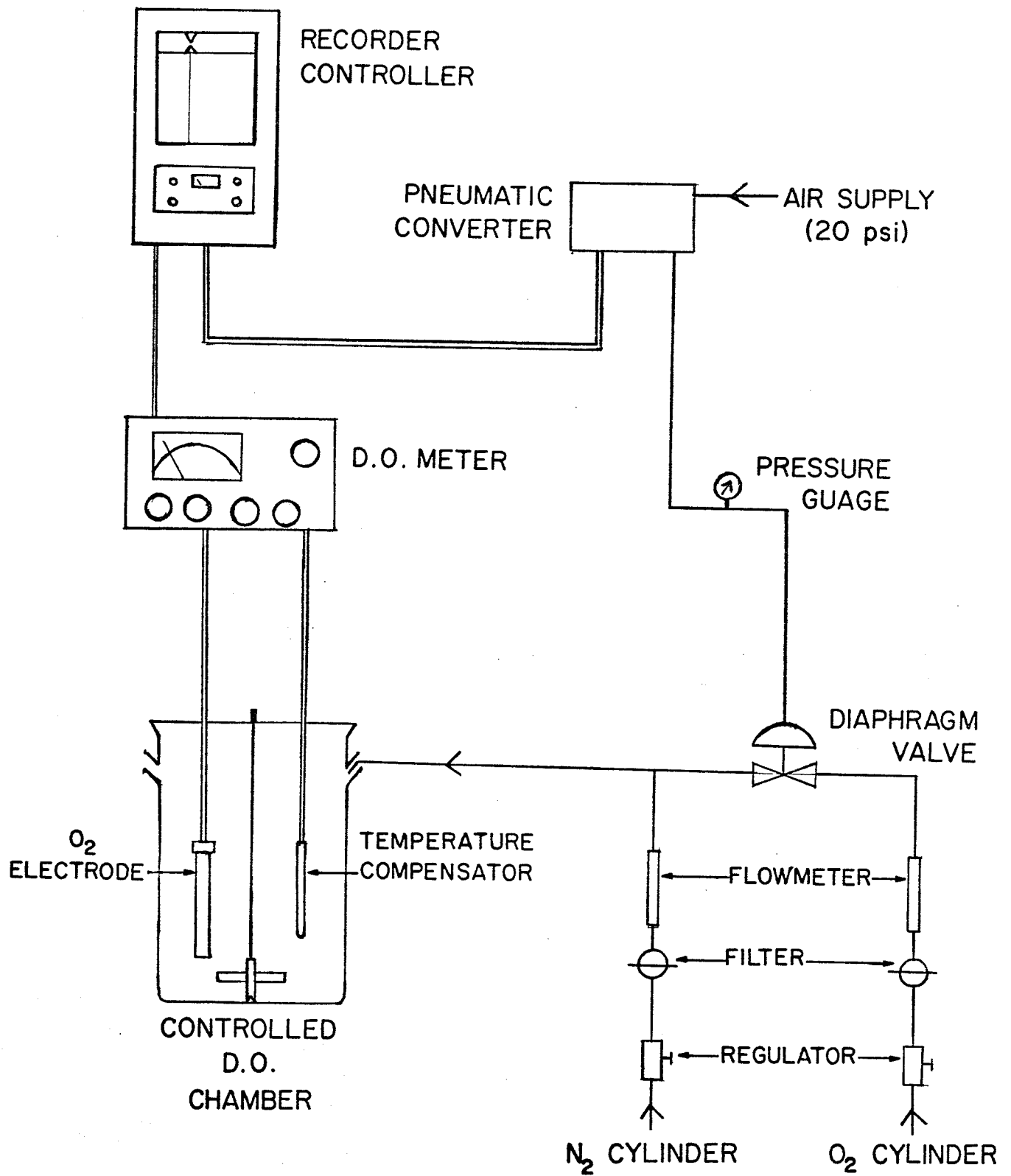
The sediment was washed in buffer once to recover trapped bacteria. The combined supernatant liquid containing the bacteria was centrifuged at high speed (16,000 x g) for 15 minutes using the Sorvall centrifuge. The sedimented bacteria were washed in buffer, spun down and resuspended. This washing procedure was done twice to ensure that all soluble compounds were removed from the cell preparation. The cells from a 20 liter lagoon sample were used to prepare 200 ml of resting cell suspension, representing a 100 fold concentration of the bacterial population present in the lagoon at the site of sampling.

In experiments where detergents were used as substrate, final suspensions were made up in distilled water rather than in buffer because inorganic ions interfere with the quantitative estimation of such compounds by the "methylene blue" method (43).

Automatic Control of Dissolved Oxygen Concentration in Stirred Microbial Resting Cell Suspensions

Automatic control of dissolved oxygen concentration in resting cell suspensions was achieved by using the Mackereth oxygen electrode as a sensing probe in an equipment setup modified after Pirt (28). The DO control system employed is represented in Fig. 3. Controlled DO levels are maintained within 1-2% of the control point settings indicating a maximum error of ± 0.16 ppm at 25°C and ± 0.26 ppm at 2°C.

Fig. 3 Equipment setup for automatic oxygen control:
a schematic showing the componentry of the
control apparatus and the fashion in which it
is assembled.



D.O. Control Equipment:

I. Model T15A dissolved oxygen meter, A15A biological oxygen electrode (Electronics Instruments Ltd. Richmond Surrey England).

The model T15A DO meter is a battery operated instrument which is housed in a steel case. It is highly portable and thus suitable for field or laboratory work. This particular model includes an automatic temperature compensator and resistance thermometer probe, which records temperature and corrects for temperature effects on DO readings. The model A15A electrode relies on the chemical reduction of oxygen which diffuses through the semi-permeable membrane covering the electrode at a suitable cathode to give a current proportional to the oxygen partial pressure. The electrode is cylindrical in shape and consists of a central lead anode which is surrounded by a porous polyvinyl chloride (pvc) membrane. The cathode is a hollow cylinder of silver and is surrounded by a polythene membrane which is permeable only to the oxygen in the water. The whole electrode is filled with a suitable electrolyte which forms a conducting path between the anode and the cathode, prevents them from making direct electrical contact.

II. Series 60, 3-Action Current-Adjusting Type (C.A.T.) control unit, (Leeds and Northrup, Sumneytown Pike, North Wales, Pa.)

In general, a Series 60 Current-Adjusting Type (C.A.T.) control system comprises an arrangement of the following major components:

- (1) A primary element sensitive to the variable quantity to be controlled.
- (2) A recorder or indicator which measures the output of the primary element. This instrument, which also contains a control setting device, signals the control unit whenever the measured variable departs from the control point setting.
- (3) A control unit which automatically produces an output current in the range 0-5 milliamps proportional to the input requirement of the controlled process.
- (4) Equipment for translating the current output of the control unit into process input. This is usually a pneumatic converter having a 1-5 milliamp input range.

III. 10970-1 Electro-Pneumatic Converter (Leeds and Northrup, Sumneytown Pike, North Wales, Pa.)

The electro-pneumatic converter operates by converting current from the control unit to corresponding output air pressure.

IV. Pneumatic Control Valve (Research Control Valves, Tulsa, Okla., U.S.A.)

This is an air operated valve which is controlled by the output pressure of the electro-pneumatic converter. This valve regulates the input to the controlled process.

V. Ancillary Equipment

- (1) Oxygen cylinder, regulator, filter and gas flowmeter-supplies oxygen input to control system, the flow of which is regulated by the pneumatic diaphragm valve.
- (2) Nitrogen cylinder, regulator, bacterial filter and gas flowmeter-supplies nitrogen input to control system at a constant flow rate.
- (3) Water-jacketted jar, temperature regulating unit and pump, mechanical stirrer, used for temperature control and agitation of resting cells suspension during the course of an experiment.

Substrates

The scope of this study was limited to detergents of the linear alkylate sulfonate (LAS) type; plus benzoate, salicylate and butyrate. These organic compounds were chosen as they were known to require oxygen for their degradation.

Commercial LAS Composite SDA 1-1, (Monsanto Chemical Co., St. Louis, Mo.) prepared by sulfonation of a composite linear alkylate, chain length predominately C_{11} , C_{12} , C_{13} . Liqui-nox, (Alconox Inc., N.Y.) ; consists of anionic and non-ionic agents. The anionic agents are of the linear alkylate sulfonate type and the non-ionic portion consists

of a linear chain octyl-phenol ethylene oxide condensate.

Salicylic acid.

Benzoic acid.

Butyric acid.

Salicylic acid, benzoic acid and butyric acid were of the highest commercial grade available.

Standard Experimental Procedure

The following steps were applied as standard procedure in all experimental work carried out.

(1) A sample of lagoon water was taken from the Air-Aqua cell during the summer, or from the Surface Aerator cell during the winter.

(2) A resting cell suspension of 100X concentration was prepared.

(3) A resting cell suspension was used for 3 trials:

a. Aerobic trial - a stirred sample open to the air.

At 1 atmosphere air pressure this represents dissolved oxygen levels of 13.40 ppm at 2°C and 8.11 ppm at 25°C.

b. Controlled DO trial - a test at a particular dissolved oxygen tension using control equipment (see page 18 and Fig. 3).

c. Anaerobic trial - an anaerobic system, a serum vial flushed with argon to remove residual oxygen.

Each controlled DO test was done in coincidence with an aerobic and anaerobic trial using the same bacterial cell

suspension and temperature of operation. This experimental design allowed for an examination of the catabolic activity as a function of the oxygen tension since the oxygen concentration was the only variable in the system.

(4) Experiments involving mesophiles (summer samples) were carried out at 25°C, whereas experiments involving psychrophiles (winter samples) were done at 2°C.

(5) Substrates (see page 24) were added to cell suspensions in amounts to make final concentrations of approximately 100 ppm.

(6) Samples were taken periodically and analyzed (see page 26) for residual substrate.

Analytical Methods

(1) LAS and Liqui-nox:

The quantitative analysis of detergents was carried out by the standard "methylene blue" method (43). This method involves the solubilization by detergent action of methylene blue in chloroform and measurement spectrophotometrically of the amount of methylene blue removed to the chloroform fraction. A percent transmittance calibration curve prepared from ABS (tetrapropylene alkyl benzene sulfonate), served as the reference standard.

Detergents measured in terms of ABS are more precisely

reported as "MBAS" (methylene blue active substance).

Slight variations in the linear chain length of different detergents do not alter significantly the detergent action and no corrections were made for variations in chain length.

(2) Salicylic acid:

The quantitative analysis of salicylic acid was done by the ferric nitrate method (41). This is a colorimetric test in which a violet color is produced by the salicylate and the ferric ion. The color is pH sensitive, maximum color developing at pH 3.5; solutions over pH 7.0 fade rapidly. The test showed a linear response for solutions containing between 0-100 ppm salicylate. Compounds which have been shown to interfere with this determination include: ascorbic acid, maltol, 2-hydroxyisophthalic acid, methyl-ethyl acetoacetate (24, 41, 42).

(3) Benzoic acid:

The quantitative estimation of benzoate was done by a gas liquid chromatographic method utilizing a Varian Aerograph Model 2100, equipped with a flame ionization detector (F.I.D.). Samples containing benzoate were collected periodically during an experiment, frozen and then lyophilized to remove water (38). Solid benzoate was resolubilized in 0.7 ml. petroleum ether and the trimethylsilyl derivative prepared by the addition of 0.3 ml of Tri-Sil (Pierce

Chemical Co., Rockford, Ill.). Samples were allowed to stand in air tight plastic stoppered glass vials for two days to ensure complete derivatization and were subsequently analyzed on a 5 ft. by 3/16 in. internal diameter stainless steel column packed with Chromosorb W 60/80 mesh with a three percent (3%) SE-30 coating.

Operational characteristics:

Gas flows (a) H_2 -35 ml/min.
(b) Air-350 ml/min.
(c) He-50 ml/min.

Temperatures (a) Inlet-155°C
(b) Column-110°C
(c) Detector-160°C
-12

Sensitivities (a) Range-10
(b) Attenuation -128

(4) Butyric acid:

Butyric acid was estimated by gas solid chromatography (see benzoate) using a pyrex column 6 ft. by 1/8 in. packed with Chromosorb 101 80/100 mesh (Chromatographic Specialties, Brockville, Ont.).

The use of Chromosorb 101 as a column packing permitted the direct analysis of aqueous butyrate samples; water does not bind to this column material nor does it give an appreciable response on a F.I.D.

Operational characteristics:

Gas flows (a) H_2 -35 mls./min.
(b) Air-350 mls./min.
(c) He-50 mls./min.

Temperatures (a) Inlet-220°C
(b) Column-180°C
(c) Detector-225°C

Sensitivities (a) Range- 10^{-12}
(b) Attenuation-16

R E S U L T S

RESULTS

Utilization of LAS and Liqui-nox as a Function of Climate

In the present study psychrophilic populations recovered from an aerated lagoon cell were employed. It was found that no degradation of LAS or Liqui-nox could be achieved over a 7 day test period when employing resting cell suspensions maintained at temperatures of 2°C or 15°C. It was decided to examine detergent breakdown by growing cell suspensions of these psychrophilic organisms. The suspension used was simply an untreated sample of lagoon water. Liqui-nox and LAS were added directly to lagoon water which was controlled at a temperature of 2°C. No degradation occurred over a period of 40 days.

Utilization of LAS and Liqui-nox as a Function of Oxygen Tension

The utilization of detergents of the linear alkylate sulfonate type by a mesophilic bacterial population was found to be oxygen dependent, in agreement with the report of Halvorson and Ishaque (19). However, lowering the oxygen tension to a level as low as 0.5 ppm did not significantly alter the rates of degradation from that of the open air stirred sample.

The time-course degradation curves for LAS and Liqui-nox by mesophiles under aerobic, anaerobic and 0.5 ppm oxygen tension levels at 25°C are shown in Figs. 4 and 5.

Utilization of Benzoate as a Function of Oxygen Tension

Fig. 6 shows the standard curve for the quantitative determination of benzoate by gas chromatography using the trimethylsilyl (TMS) derivative. The linear recorder response is shown for benzoate injections in the range between 0 and 300 ng. Under the operational conditions employed (see page 27) the retention time was 12.7 minutes.

It was found that benzoate was utilized under aerobic conditions by a psychrophilic lagoon population from an aerated cell. Under anaerobic conditions no breakdown of benzoate occurred. Experiments carried out at 1 ppm oxygen tension showed analagous utilization rates to those trials under fully aerobic conditions. In both the fully aerobic

Fig. 4 Utilization of LAS at 25°C under aerobic, anaerobic and 0.5 ppm
 oxygen tension levels by a resting cell suspension of mesophilic
 bacteria from an aerated lagoon cell.

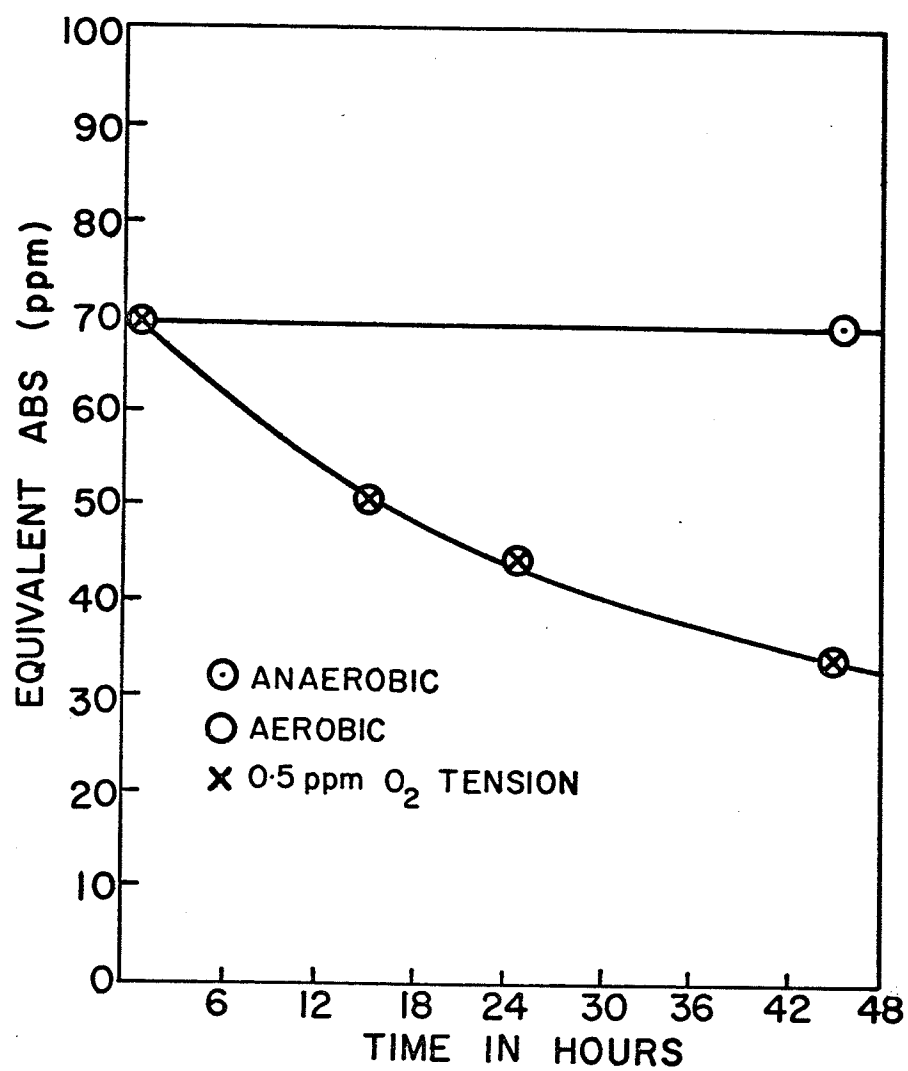


Fig. 5 Utilization of Liqui-nox at 25°C under aerobic, anaerobic and 0.5 ppm oxygen tension levels by a resting cell suspension of mesophilic bacteria from an aerated lagoon cell.

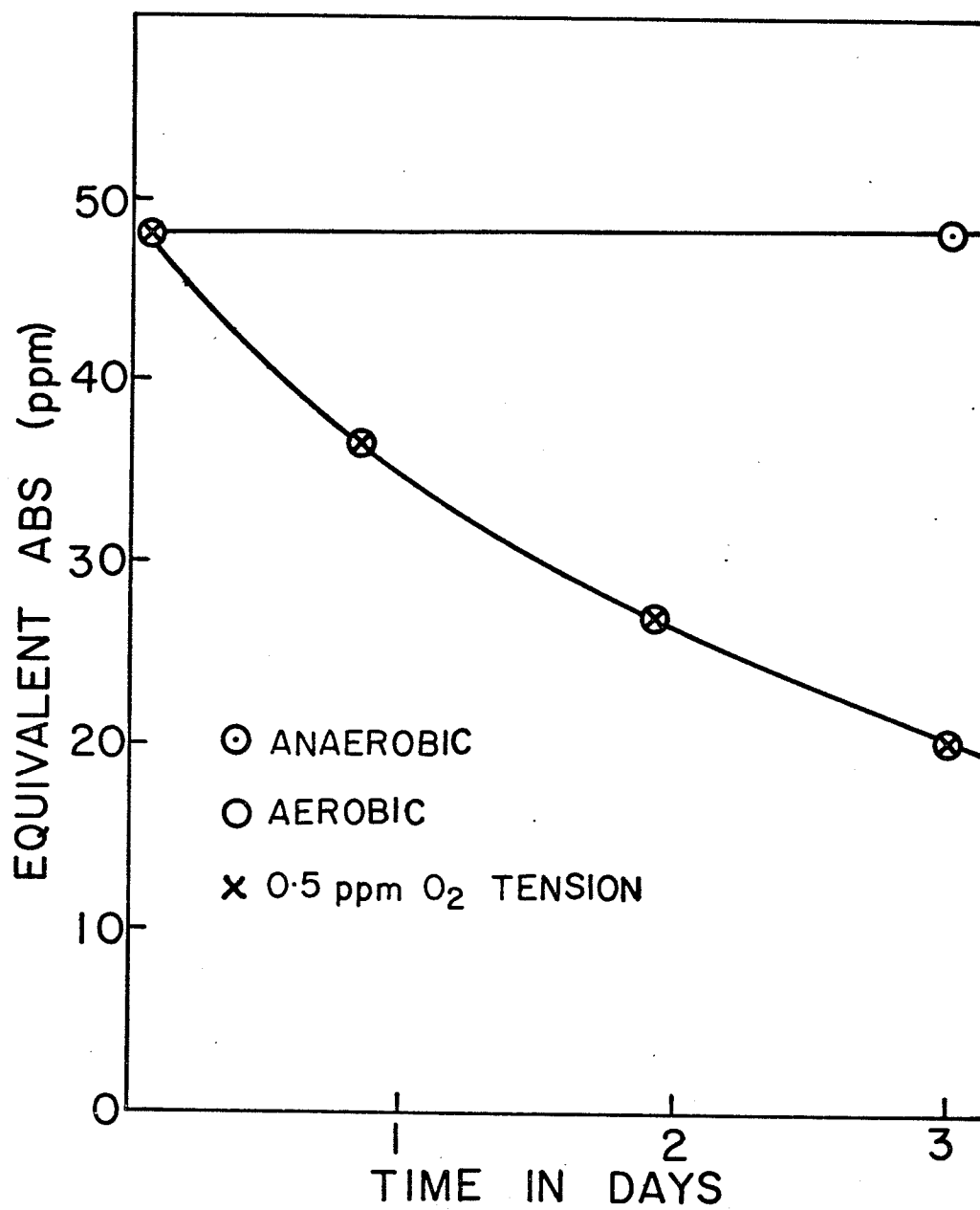
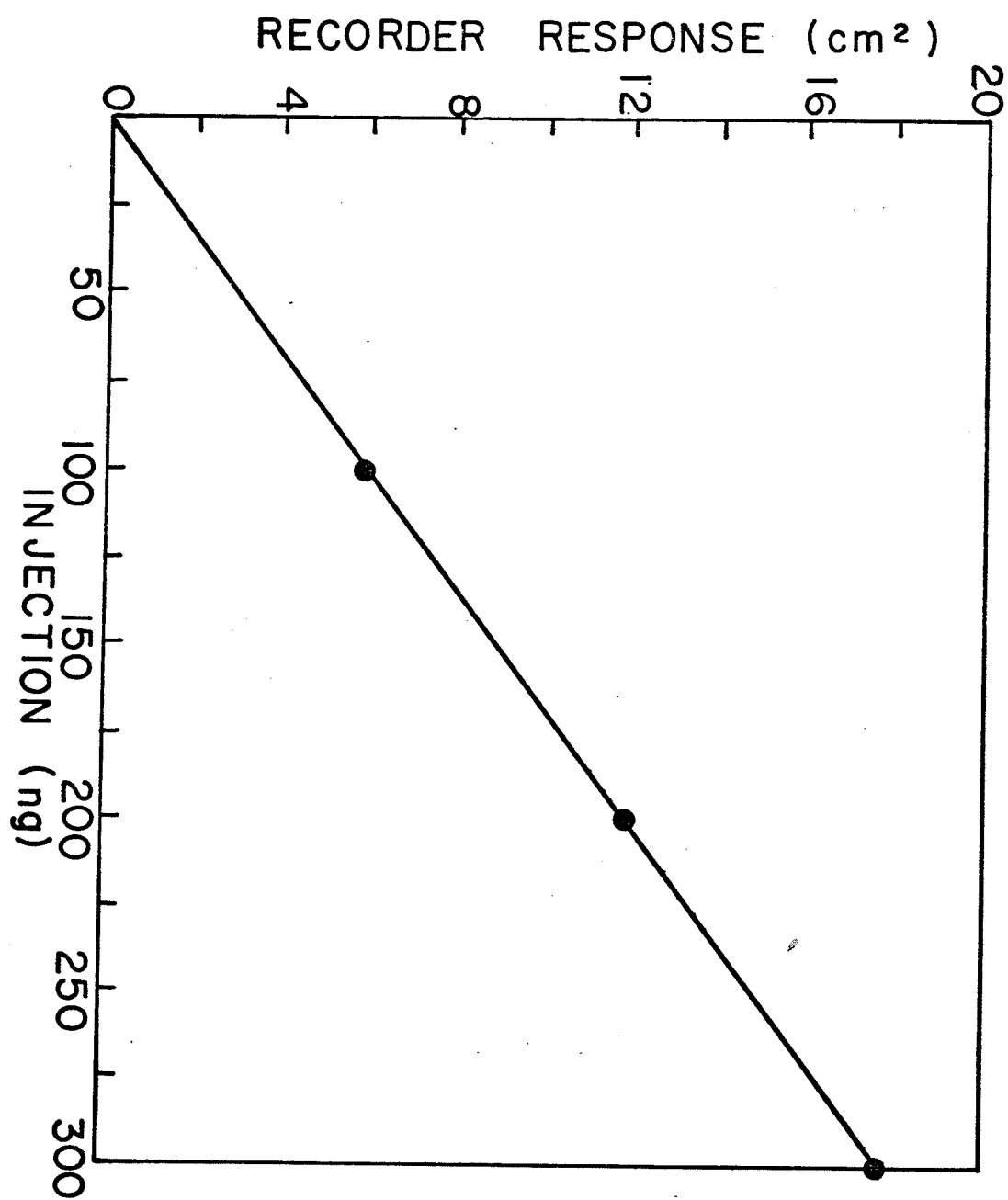


Fig. 6 Linearity plot of peak area vs. sample size (in nanograms)
 of benzoate.



and 1 ppm oxygen tension cases there was an indication of a lag period (approx. 2 hrs.) plus there was a slowing down in the degradation rate after 12 hours.

The time-course degradation curves for the utilization of benzoate under aerobic, anaerobic and 1 ppm oxygen tension at 20°C is given in Fig. 7.

Utilization of Salicylate as a Function of Oxygen Tension

It was found that salicylate was utilized under aerobic conditions by mesophilic lagoon bacteria. Under anaerobic conditions no breakdown of salicylate occurred.

When the oxygen tension level was controlled at 1.5 ppm the rate of salicylate utilization was identical to that of the fully aerobic sample. At 1 ppm oxygen tension the degradation rate was slightly lower. Further reduction of the oxygen tension to 0.5 ppm revealed a degradation rate which was markedly lower than that of the fully aerobic trial. In all instances where utilization of salicylate occurred a lag period of approximately 8 hrs. was found.

Degradation rate curves for the utilization of salicylate by mesophiles under aerobic, anaerobic, 1.5 ppm, 1 ppm and 0.5 ppm oxygen tension levels at 25°C are shown in Fig. 8, 9 and 10.

Fig. 7 Utilization of Benzoate at 2°C under aerobic, anaerobic and 1 ppm
 oxygen tension levels by a resting cell suspension of
 psychrophilic bacteria from an aerated lagoon cell.

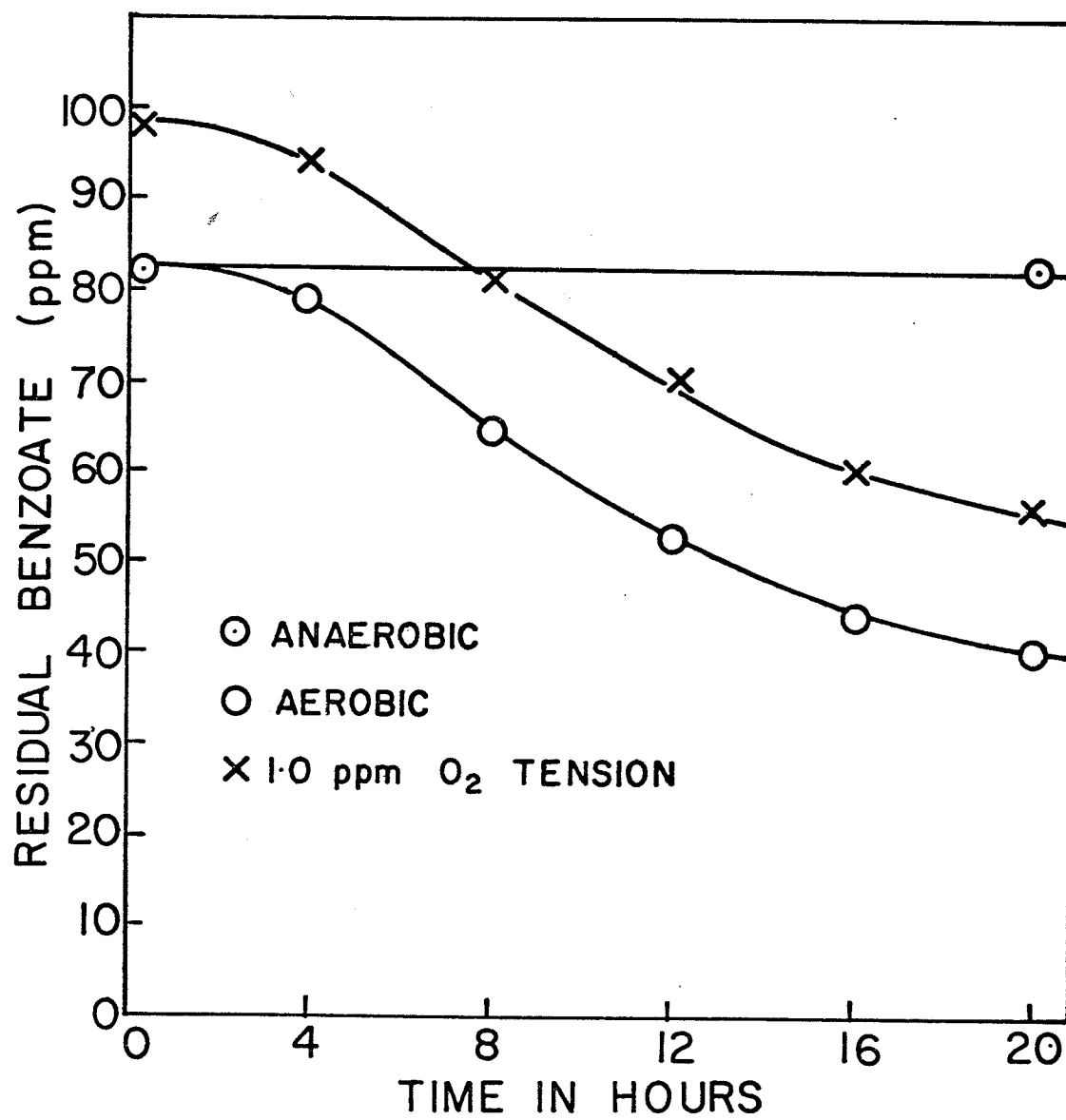


Fig. 8 Utilization of Salicylate at 25°C under aerobic, anaerobic and 1.5 ppm oxygen tension levels by a resting cell suspension of mesophilic bacteria from an aerated lagoon cell.

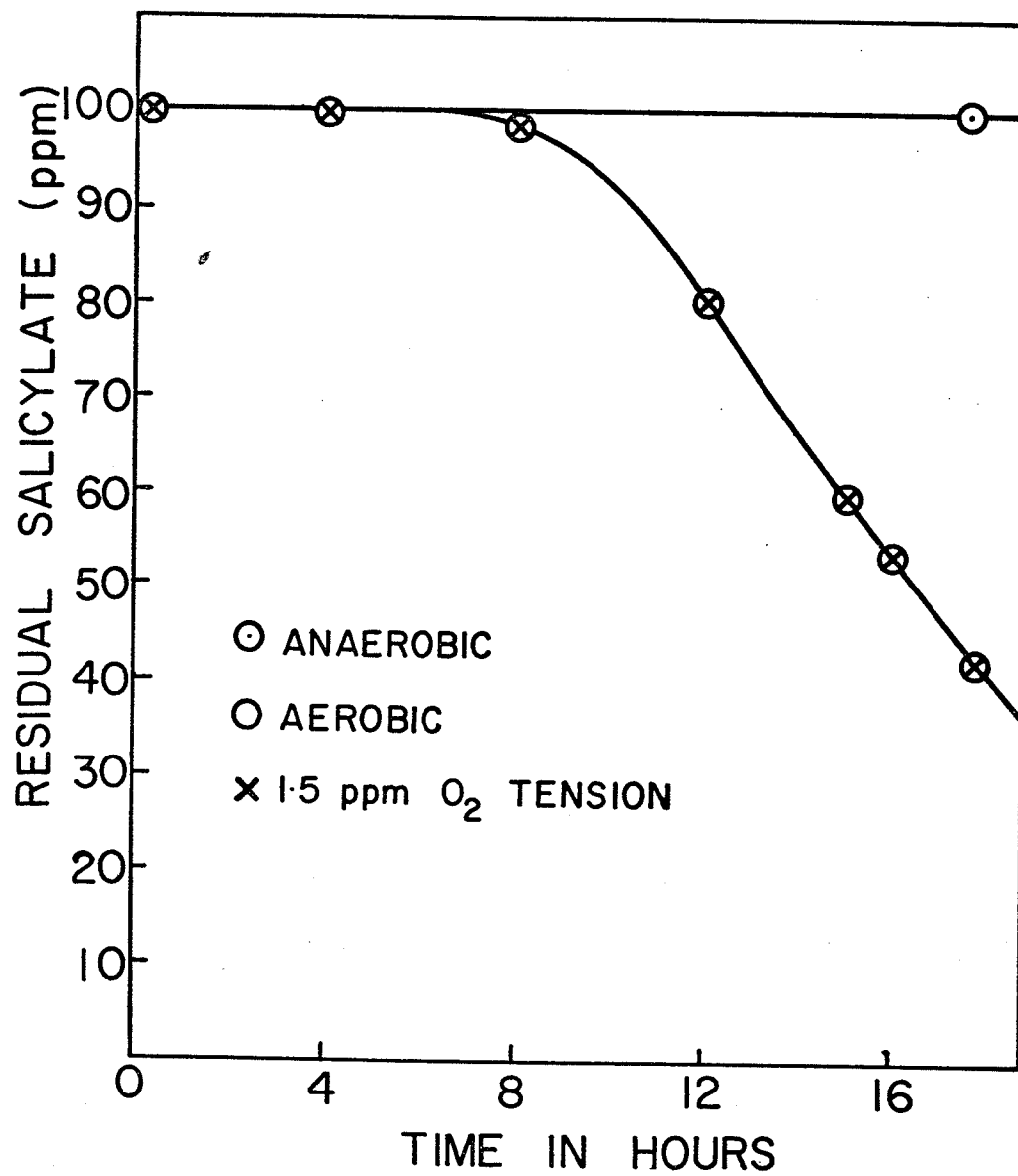


Fig. 9 Utilization of Salicylate at 25°C under aerobic, anaerobic and 1 ppm
oxygen tension levels by a resting cell suspension of mesophilic
bacteria from an aerated lagoon cell.

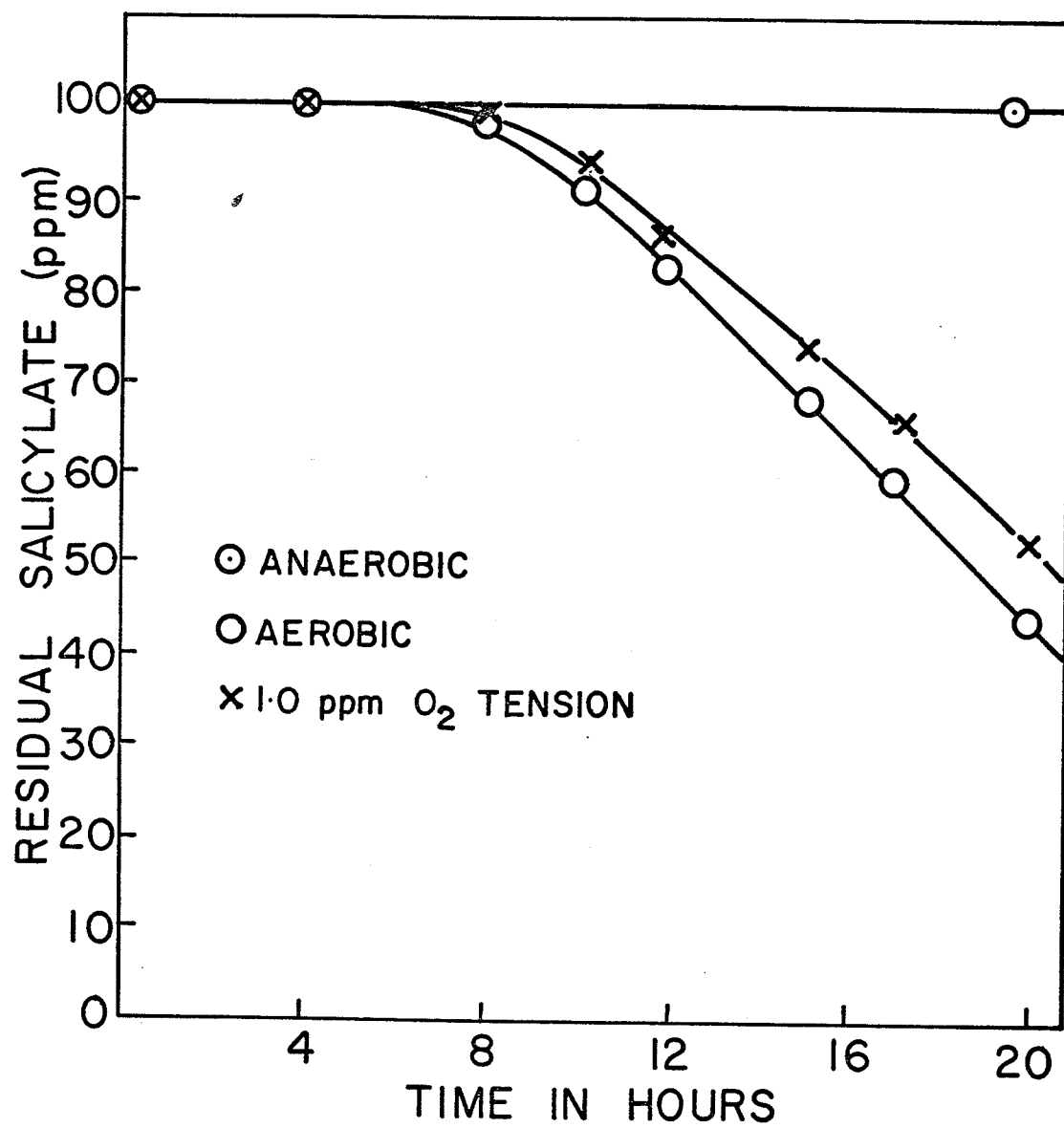
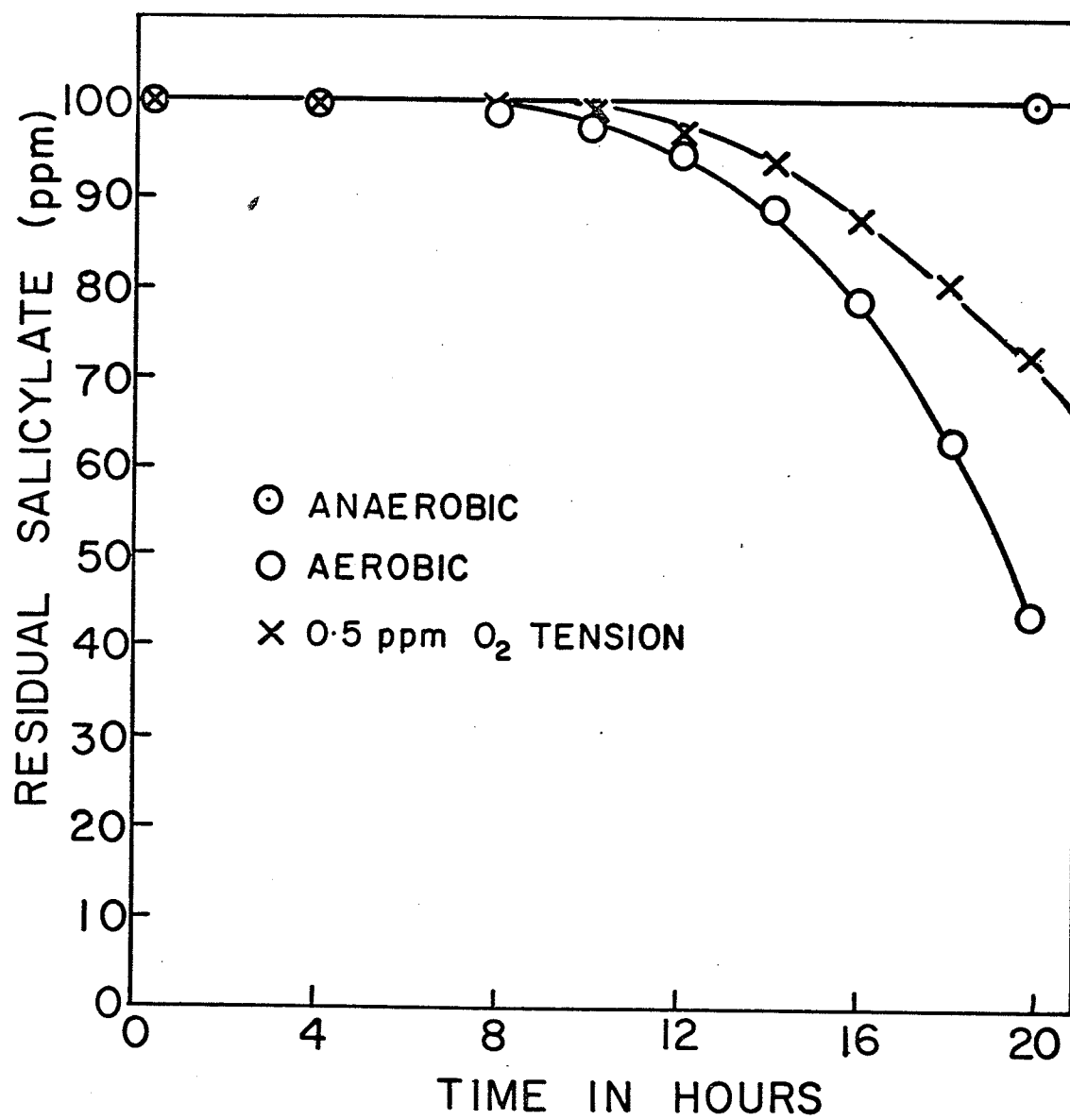


Fig. 10

Utilization of Salicylate at 25°C under aerobic, anaerobic and 0.5 ppm oxygen tension levels by a resting cell suspension of mesophilic bacteria from an aerated lagoon cell.



Utilization of Butyrate as a Function of Oxygen Tension

Fig. 11 shows the standard curve used for the quantitative determination of butyrate by gas chromatography. The recorder response for butyrate was found to be linear in the range between 0 and 250 ng. Under the operational conditions employed (see page 28) the retention time was 4 minutes.

Butyrate was utilized under aerobic conditions by the mesophilic lagoon population employed. Butyrate was not metabolized in the absence of oxygen. At the level of 1 ppm oxygen tension the rate of butyrate utilization appeared to exceed that of the aerobic sample. This was not the usual case, as all other substrates examined showed reductions in degradation rates with reductions in oxygen tension. However the probability that this result may be an artifact cannot be discarded because it was the observation of a single experiment.

The degradation rate curve for the utilization of butyrate by mesophiles at 25°C under aerobic, anaerobic, and 1 ppm oxygen tension levels is given in Fig. 12.

Fig. 11 Linearity plot of recorder response (integrator units) vs. sample size
 (in nanograms) of butyrate.

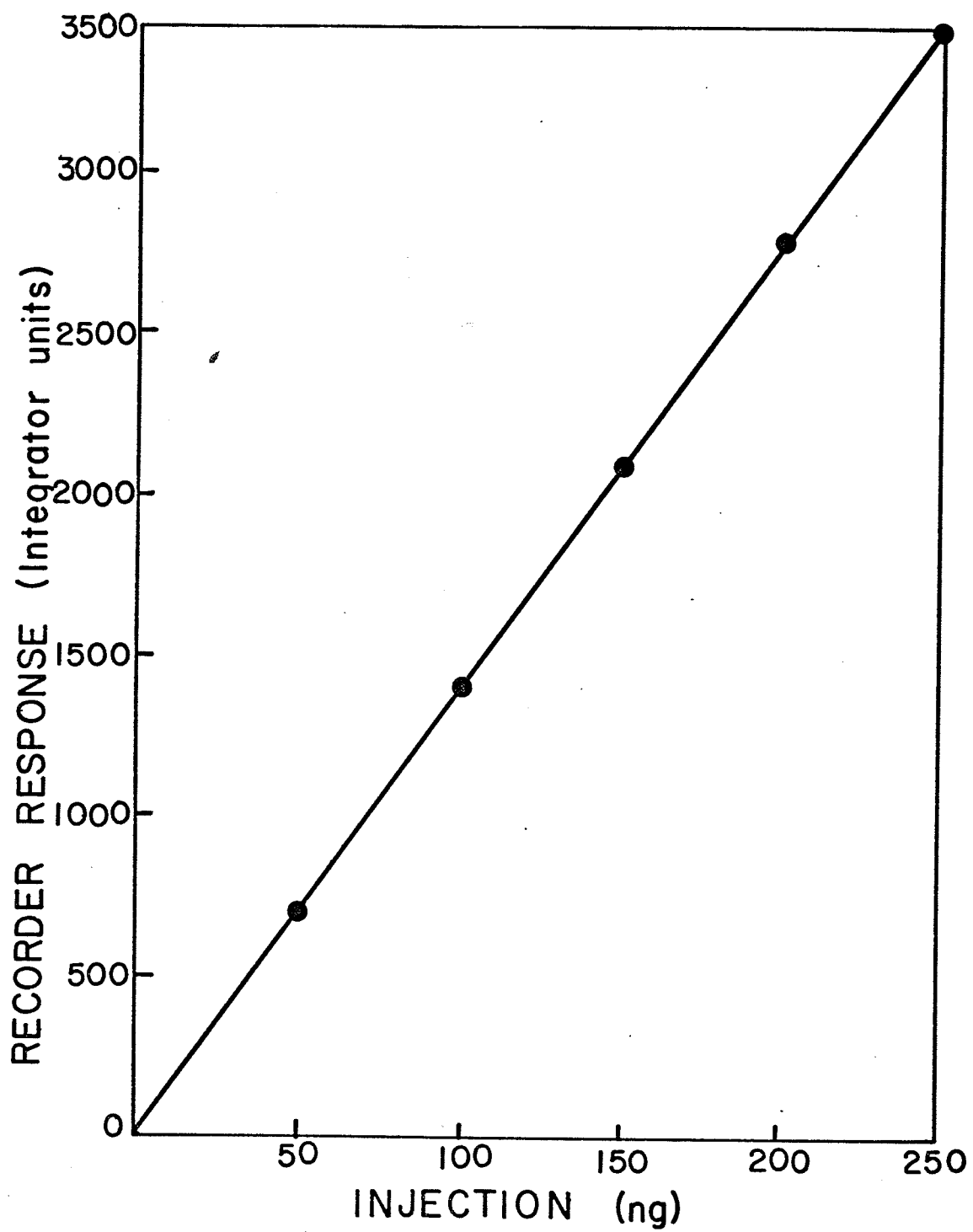
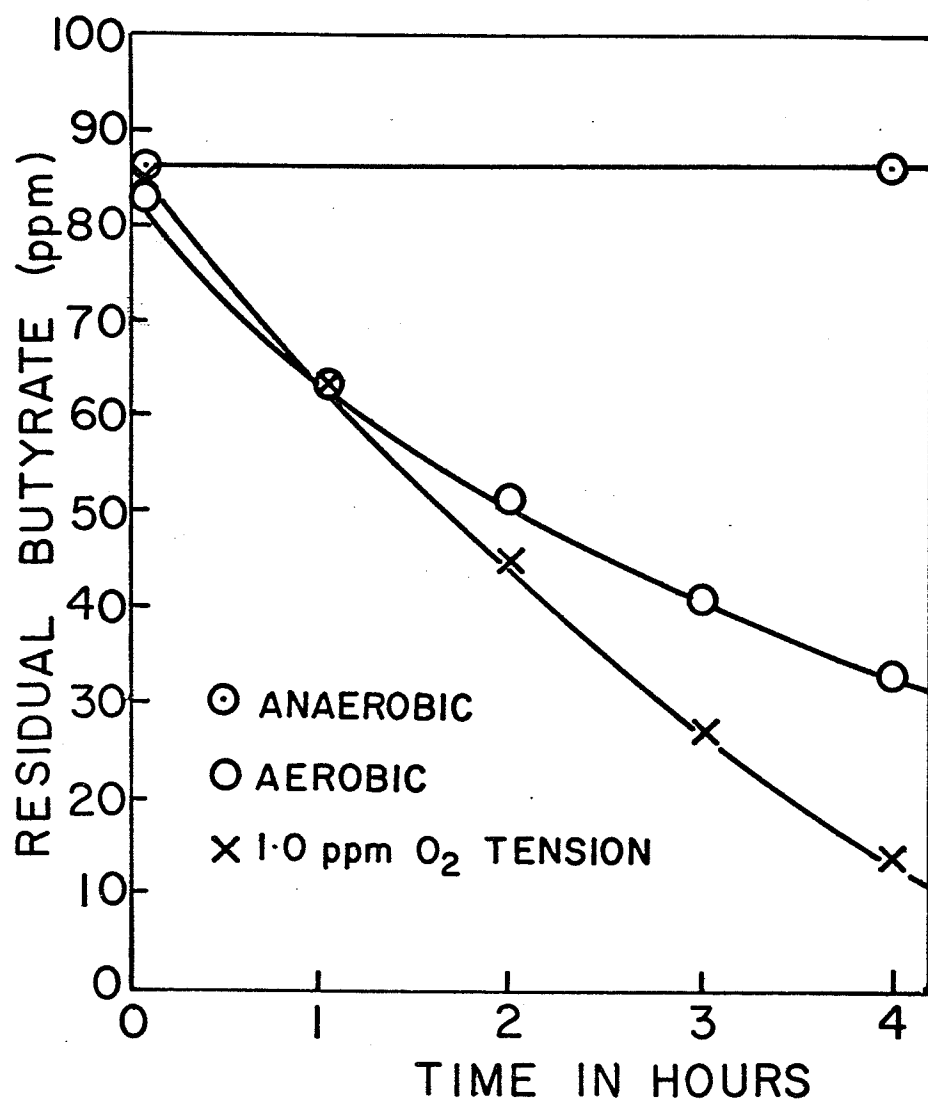


Fig. 12 Utilization of Butyrate at 25°C under aerobic, anaerobic and 1 ppm
oxygen tension levels by a resting cell suspension of mesophilic
bacteria from an aerated lagoon cell.



D I S C U S S I O N

DISCUSSION

Since 1965 (2, 3), linear alkylate sulfonate detergents have come into prominence replacing the formerly used microbially resistant ABS anionic detergents and now represent a significant proportion of the MBAS load entering sewage lagoon disposal units. In working with mixed resting cell preparations from conventional (naturally aerated) lagoon systems, Halvorson and Ishaque (19) have shown the ability of mesophilic populations to degrade a number of LAS-type detergents under aerobic test conditions; the degradation rate falling with reduced temperatures until no utilization could be observed at 2°C. As well, the aerobic dependency of the detergent breakdown was amply demonstrated by the inability of Liqui-nox to be utilized under anaerobic conditions. In the same study, mixed resting cell preparations of psychrophiles derived from a lagoon under ice cover (anaerobic system) were shown to be unable to degrade LAS-type detergents under aerobic test conditions at 2°C.

Under ice cover, an environment suitable only for the development of strict anaerobic and facultative anaerobic psychrophiles prevails. The possibility that a psychrophilic population developing under aerobic conditions might have the

metabolic capability to utilize LAS-type detergents remained uninvestigated.

The preliminary investigations entered upon in this project were therefore designed to assess the ability of psychrophiles from an artificially aerated lagoon (aerobic system) to utilize LAS and Liqui-nox. Should utilization have occurred, studies on affects of DO tension on the catabolic rates involved would have been pursued. However resting cell suspensions of these organisms were unable to effect detergent breakdown under aerobic conditions at 2°C and 15°C over a 7 day period. The 15°C test condition was employed in addition to the 2°C trial in an attempt to initiate detergent utilization if in actuality breakdown was not occurring at the lower temperature, or to accelerate the degradation should utilization be occurring at an undetectably slow rate. As breakdown did not occur at either temperature the experimental findings serve to demonstrate that the aerobically developed psychrophiles lack the capability to degrade LAS-type detergents and that the inability to do so is not simply a temperature effect.

For final confirmation that detergent breakdown was not occurring by aerobic psychrophiles, an experiment of long term duration under growing cell conditions was carried out.

In this trial an untreated winter sample of lagoon water from an artificially aerated cell was employed, as by its nature it contained the organic nutrients and growth factors required for the sustenance of the psychrophilic population. The experiment provided growing cell conditions but differs from the lagoon situation which may be most closely compared to a continuous culture operation whereas the experimental setup was in effect of batch culture design. Over a period of 40 days at 2°C no degradation of added LAS or Liqui-nox could be detected. A trial at 15°C was not attempted as the possibility of the development of a mesophilic population was evident. The findings presented here may be viewed as further evidence that LAS-type detergents are not metabolized by psychrophiles during the winter period in either conventional or artificially aerated lagoons.

The major segment of this manuscript involved the investigation of the role of oxygen as a catabolic regulator with specific reference to the stabilization of LAS-type detergents by artificially aerated lagoon systems. The experiments were designed in an attempt to determine the minimum DO levels (threshold levels) at which the utilization of such detergents as well as benzoate, salicylate and butyrate could proceed at maximum efficiency.

The threshold oxygen level as defined here refers to

that DO level above which the metabolic rate of utilization does not increase, and below which the rate of utilization declines. This definition assumes that all other parameters in the system remain constant. Threshold values were determined by comparing an experimental trial at a particular DO level to a trial performed simultaneously at full aerobic tension using the same bacterial resting cell suspension. As well, in all cases an anaerobic control was used to ensure that the substrate was not being dissimilated in the absence of oxygen.

In practice, if a trial at a particular oxygen level displayed the same rate of substrate disappearance as a fully aerobic sample and any reduction in the DO tension from this point resulted in a decreased degradation rate then this former DO level was termed the threshold value. If a DO of 1 ppm or 0.5 ppm gave an analogous degradation rate with that of a fully aerobic trial then this was considered to be a low enough level that further investigation was not of practical importance. The experimental findings are therefore referred to as threshold DO levels ≤ 1 ppm or ≤ 0.5 ppm whichever applies.

The detergent trials with mesophilic lagoon organisms showed threshold values of ≤ 0.5 ppm., see Figs. 4 and 5. The benzoate trials with psychrophiles showed threshold DO

levels of ≤ 1 ppm, see Fig. (7). The salicylate experimental trials with mesophilic organisms disclosed a threshold value of ≤ 1.5 ppm, see Figs. (8, 9, 10). The trials with butyrate, employing mesophiles, showed a threshold value of ≤ 1 ppm, see Fig. (12). The anaerobic controls, in all instances displayed no substrate disappearance.

The failure of LAS-type detergents, benzoate, salicylate and butyrate to be degraded anaerobically is not unexpected; the results being consistent with the most predominant and widespread mechanism of aromatic ring cleavage (21, 44, 8, 37, 7, 48) and the probable regeneration of reduced pyridine nucleotides resulting from the metabolism of straight chain aliphatics (25, 47) by a β -oxidation scheme.

Microbial attack upon aromatic compounds is characterized by a dependency upon induced enzyme formation unless the organism has been pre-cultured in the presence of that particular aromatic or related compound (9, 10, 16). The findings in the present study are consistent with this observation. Salicylate exhibited a well defined initial time interval (induction period) during which utilization did not occur, see Fig. 8. In the case of benzoate there is an indication of a lag period however there is not enough data to substantiate this, see Fig. 7.

An unexpected observation occurred with respect to

butyrate metabolism. The disappearance rate at 1 ppm DO level was faster than the fully aerobic sample during the majority of the test period, see Fig. 12. As the results presented are simply the observations of a single experiment the possibility of an artifact resides. However the slope of the substrate disappearance curve at 1 ppm DO is appreciably steeper than that at full aerobic tension and this would indicate that the experimental finding is valid. As utilization was not seen to occur under anaerobic conditions, the possibility of a fermentative pathway accounting for the observed increased degradation rate at lower oxygen tensions may be discounted.

A possible explanation of the phenomena may come out of studies as reported by Harrison (20) and White (49); that in some instances the response to lowered DO tensions may in fact result in an increase in respiration rate. Harrison (20) suggests that the increased oxygen uptake as witnessed in certain low oxygen situations may be due to a decreased ATP production yield possibly due to an alternate electron transport system pathway or to a partial uncoupling of oxidative phosphorylation. In any event such an elevated respiration rate would be indicative of higher catabolic rates in the organisms and consequent higher rates of substrate disappearance.

"The selection of an experimental system immediately imposes constraints upon the work to be done and to a greater or lesser extent pre-determines the results obtained from a study" (6). In other words, an evaluation of the threshold DO data and its significance with respect to lagoon performance can only be made after due consideration is given to parameters not accounted for by the experimental system. Foremost is the consideration that the lagoon is a growing cell state, whereas experiments were carried out on resting cell preparations. The oxygen demands of a growing cell system may be quite different than that of a resting cell suspension.

Johnson (23) and Button (6) have shown that oxygen concentration can become a limiting factor with respect to microbial growth. However Johnson (23) in work with Candida utilis growing on acetate found a K_m (Michaelis constant) for growth with oxygen of 1.3×10^{-6} M (0.04 ppm) a very low oxygen tension. Button et al (6) in work with Torulopsis utilis growing on glycerol found a K_m for growth with oxygen of 1.4×10^{-5} M (0.45 ppm). The results indicate that for these particular organisms and substrates oxygen tensions exceeding 1 ppm should not be growth limiting. However it is difficult at the present time to speculate upon levels of oxygen which would become growth limiting

in an actual lagoon situation where one finds a mixed microbial population and a wide diversity of organic nutrients.

Another possible effect of DO tension not accounted for in this study is lagoon population shifts. Changes in predominating species at different DO tensions have been suggested by Wuhrmann (51). As well, it is essential to appreciate that air released into lagoon cells may serve other significant functions aside from a metabolic role. The bubbling of air into the Air-Aqua installation and the churning action of the Surface Aerator cell in all probability contribute to the mixing efficiency of these operations. Also, high degrees of aeration are known to alleviate nuisance odor problems associated with anaerobic treatment processes.

Due to the nature of the limitations on the experimental system, the threshold DO values reported here cannot be justifiably used as an absolute index of the minimal oxygen requirements for efficient lagoon performance. The data may however serve to indicate some aspects of lagoon oxygen requirements and to delineate some possible lines of future investigation in this area.

The resting cell studies with oxygen dependent substrates of the LAS-type and representative aromatic and aliphatic moieties indicated a level of 1.5 ppm oxygen tension as being more than adequate for their optimum utilization. It would

appear that the value of 1.5 ppm DO is above the regions of oxygen limiting growth that have been reported (23, 6). Should this be in fact the situation in the lagoon, then the results would indicate that maintenance of higher DO values than 1.5 ppm with respect to LAS-type detergent stabilization in lagoons may be unwarranted. The value of 1.5 ppm DO falls within the range of minimum DO levels (0.5 ppm to 2.0 ppm) that have been recommended (45) for the operation of activated sludge units and well below the operational levels in the Charleswood Sewage Lagoon (15)

Before any final statements can be made concerning DO levels of lagoon operation more substrates indigenous to lagoon situations require testing. Also the use of growing cell conditions may be warranted for conclusive results. One possible means of testing the effect of DO on a large number of substrates native to a lagoon, is by using a continuous flow reactor system under conditions approximating a steady state (36). By varying the DO concentration of the system, one could note the possible affects on bacterial growth, changes in predominating species or upon efficiency of BOD removal. In effect such a system would closely resemble an actual lagoon situation making results obtained more directly applicable to solving problems of waste water stabilization.

S U M M A R Y

SUMMARY

The results of experiments investigating the degradation rates of some LAS-type detergents and representative aliphatic and aromatic moieties thereof under various DO tensions by microflora indigenous to artificially aerated lagoons are presented.

1. LAS and Liqui-nox were not degraded by psychrophiles under aerobic conditions employing either resting cell preparations at 2°C and 15°C or growing cell preparations at 2°C.
2. Degradation of substrates by resting cell suspensions under various DO conditions revealed the following minimum oxygen requirements (threshold levels) for optimum utilization.

- (a) LAS and Liqui-nox by mesophiles at 25°C ---- $\leq$ 0.5 ppm DO
- (b) Benzoate by psychrophiles at 2°C ---- $\leq$ 1.0 ppm DO
- (c) Salicylate by mesophiles at 25°C ---- $\leq$ 1.5 ppm DO
- (d) Butyrate by mesophiles at 25°C ---- $\leq$ 1.0 ppm DO

3. None of the substrates investigated, LAS, Liqui-nox, benzoate, salicylate and butyrate were metabolized under anaerobic conditions.

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